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RESEARCH ARTICLE

Stability Indicating Method Development Degradation Studies and Validation of Cefpodoxime Proxetil by RP-HPLC Method

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ABSTRACT

A new RP-HPLC method has been developed and validated for the stability studies of Cefpodoxime proxetil and detection of isomers. Degradation of products is produced through the process of degradation such as acid, base, heating, respectively. The experimental separation was carried out using Hypersil C_{18}(250*4.6,5µm particle size) BDS column with a flow rate of 1.2ml/min and PDA detector to detect the wave length at the 235nm.The mobile phase consists of Acetonitrile and mixed phosphate buffer of pH 6.8 in the ratio of 65:35.validation parameters of precision and accuracy data for Cefpodoxime proxetil and isomer of standard deviations(SD) obtained were 0.002939388 and 0.007332121.The linear regression analysis data for the calibration curves for both the plots showing linear relationship with the R^2 values of 0.9999 for CP and 0.998 for isomer with the concentration range of 50-200 µg/ml. The LOD and LOQ were estimated as 3.21 and 9.83µg/ml. Robustness and ruggedness trails obtained were 9.400 and 8.649, 8.304 and 9.005 for CP and isomer respectively .The standard peaks were produced by the retention time (RT) values of 9.299 and 8.204 Tailing factors were found to be below 2.The comparative studies of Assay was performed on drug and isomer to determine the standard deviation of samples and % assay were found to be 99.84% and 99.86% respectively.

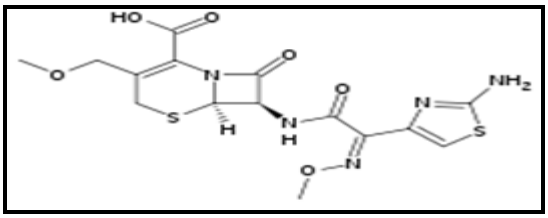
KEYWORDS

Cefpodoxime Proxetil, Isomer, Reverse- phase, HPLC, Retention time

INTRODUCTION

Cefpodoxime Proxetil is chemically 1-(isopropyl carbonyloxy) ethyl (6R, 7R)-7-[2-amino-thiazolyl) – (z) -2- (methoxyamino) acetamido]- 3-methoxymethyl-3-cephem-4-carboxylate[1], is an oral third generation cephalosporin antibiotic. It is active against most Gram positive and Gram negative organisms.

Cefpodoxime inhibits cell wall synthesis by inhibiting final transpeptidation step of peptidoglycan synthesis in cell walls. It has well established pharmacokinetic profile with absorption of 50%. It is indicated in community Acquired Pneumonia, Uncomplicated skin and skin structure Infections and Uncomplicated Urinary tract infections.



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Reported methods performed for estimation of Cefpodoxime proxetil in different dosage forms through spectrophotometric and RP-HPLC process. Literature study reveals that the drug is available in market with combinations and single doses. The structural and stereo isomers of CP can be obtained in the final preparation of products related to detect the impurities present in the drug through the degradation studies of acid, base, heating which optimize the isomers present in the standard sample solution. The stability indicating method is the effective study for comparison of the peak produced through the HPLC instrument by a dilution sample. The present study describes the drug combines with metal and forms a drug metal complex ion formation with the variation of colour through degradation process. Hence there is use and optimize to predicts that it is useful for the prominent comparison of system suitability parameters and chromatographic conditions through the mobile phases of different columns used in this studies.

EXPERIMENTAL

Materials and Reagents

Cefpodoxime proxetil is an active drug which contains purity of 99.30%, available as a gift sample from KDP labs, Kothapet, Hyderabad, Triethylamine, orthophosphoric acid, ammonium dihydrogen phosphate were purchased from and which are AR grade. Acetonitrile and methanol were HPLC grade. Water as Milli Q grade.

HPLC Instrumentation

The HPLC system consists of a Shimadzu double pump and PDA detector. The column consists of Hypersil C_{18} (250*4.6, 5µm particle size) BDS.

Chromatographic Conditions and Mobile Phase Preparation

Chromatographic separation was achieved using Hypersil BDS column. The HPLC system was operated isocratically using a mobile phase consisting of Mixed phosphate Buffer: Acetonitrile using in the ratio of 65: 35 as the mobile phase at a flow rate of 1.2ml/min at room temperature. The injection volume was taken as 20µg/ml for both standard and sample. The UV detector was set at 235nm.Then the obtained peak areas were integrated automatically using Empower 2 solution software. Peak identity was determined by knowing the retention time value.

Procedures

Preparation of Cefpodoxime Proxetil Standard

Weighed and transferred 20 mg of Cefpodoxime Proxetil into a 100ml volumetric flask dissolved and diluted to volume with diluents and 0.2 ml of above solution taken and diluted to 100ml with diluent to prepare 20µg/ml.

Preparation of Sample Solution for UV Absorption (235nm)

10mg of Cefpodoxime proxetil was accurately weighed and dissolved in 10 ml of methanol to give concentration of 1mg/ml (solution A). 1ml of solution A was further diluted and UV spectrum was recorded.

Preparation of Standard Solution

Weighed and transferred 20 mg of Cefpodoxime Proxetil into a 100ml volumetric flask dissolved and diluted to volume with diluents and 0.2 ml of above solution taken and diluted to 100ml with diluent.

Validation Study

The above method was validated as per ICH guidelines using Cefpodoxime proxetil with respect to the following parameters such as Accuracy, Precision, Linearity, LOD, LOQ, Ruggedness, Robustness, Stability, Specificity.

Accuracy

It was done by recovery study using standard addition method at 50%, 100 and 150% level; known amount of standard Cefpodoxime proxetil was added to pre-analyzed sample of Cefpodoxime proxetil and subjected them to the proposed HPLC method.

Precision

This method was proved by analyzing 6 precision and accuracy samples for 5 days. Each

batch consisted of 6 replicates of freshly prepared LQC, MQC and HQC samples. Precision is reported in terms of co-efficient of variance (% CV) over the range of quantitation for a single experiment in which standards are assayed in replicate (intraday) and for series of experiments in which standards are assayed in several experiments (interday).

Linearity

For testing linearity, six calibrations were prepared in the range of 25 -150µg/ml (25, 50, 75, 100, 125, 150 µg/ml). Standard curve was obtained by plotting area against concentration, and the calibration of linearity was done by linear regression analysis (y^2) using least square method.

Limit of Detection and Limit of Quantification

The limit of detection and limit of quantification and are estimated to signal at a ratio of 3:1 and 10:1 respectively. The theoretical values were confirmed practically by injecting dilute solutions of known concentration.

LOD = SD x 3.3/s

LOQ = SD x 10/s

Where SD is the standard deviation of the area of lowest concentration , and S is the slope of the calibration graph.

Robustness

Experimental trails obtained for resolution of Cefpodoxime proxetil and its degradation product of isomer detected in order to determine the robustness of CP and isomer. Flow rate as 1.2ml/min and λ_{max} 235nm were selected. Resolution factor was changed at two levels. Difference in the experimental conditions of peak area and the retention time were noted at change in the analytical parameters.

Ruggedness

The trails obtained for resolution of Cefpodoxime proxetil and its degradation in order to determine the ruggedness of CP and isomer. The RT value of CP was above the isomer.

Table 1: Results of Accuracy (% Recovery)

Sr. No	Cefpodoxime Proxetil
% CV	0.070
Amount Recovery	147.89
% Recovery	98.59

Table 2: Results of Precision

Precision and Accuracy	% SD	% CV	% Accuracy
Cefpodoxime proxetil	0.0029 39388	3.26	91.09%
Isomers	0.0073 32121	8.16	89.8%

Table 3: Linearity of CP

Concⁿ µg/ml	Linearity and Range			
	Area Ratio (Area of Analyte/ Area of IS)			
	RT 1	Area	Height	%Area
25	8.947	733639	47005	50.673
50	8.975	1471712	93604	50.474
75	8.994	2276636	143815	50.528
100	9.004	3062293	198055	50.601
125	8.987	3759620	232866	50.567
150	9.012	4515808	277152	50.454

Table 4: Linearity Parameters

CC	Slope	Regression (r2)
Linearity 1	15135	0.999
Linearity 2	29724	0.998

Calculations

Limit of Detection and Limit of Quantification

LOD = 3.21µg/ml

LOQ = 9.83µg/ml

Table 5: Results and Discussion

S.No.	Cefpodoxime Proxetil	Isomer
Retention Time	7.116	8.257
% Recovery	99.38	
% Assay	99.84	
Precision	3.26	8.16
Accuracy	91.09%	89.08%
Linearity	30271	14861
Regression	0.999	0.998
LOD	3.21	
LOQ	9.83	

Method Development Optimized Trail

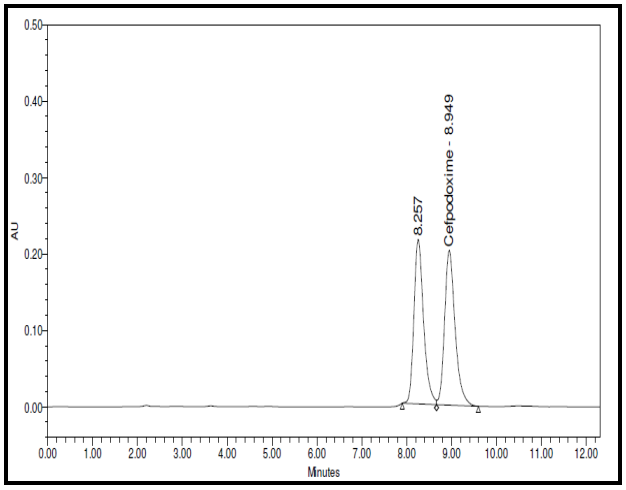

Figure 1: Chromatogram of optimized trail 5

Degradation Studies

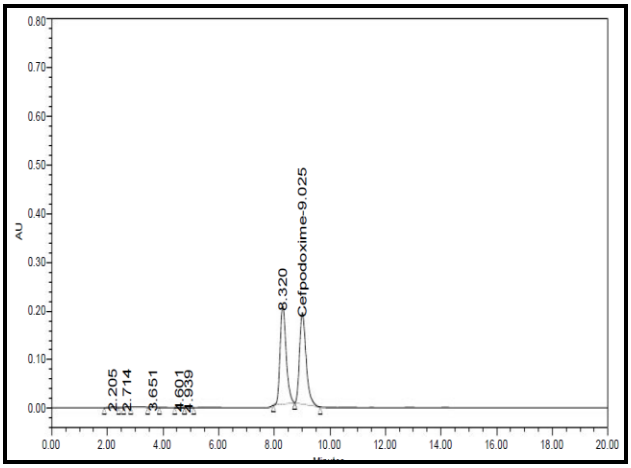

Figure 2: Chromatogram of degradation

Validation Parameters

Accuracy

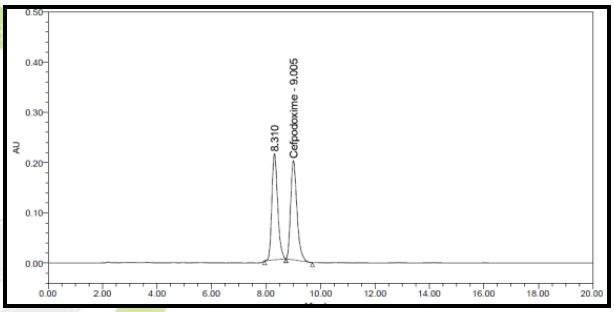

Figure 3: Chromatogram of Accuracy at 50%

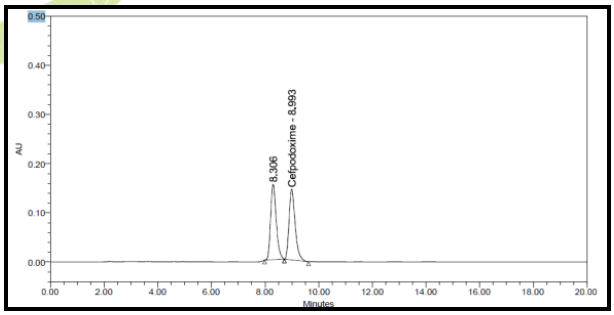

Figure 4: Chromatogram of Accuracy at 100%

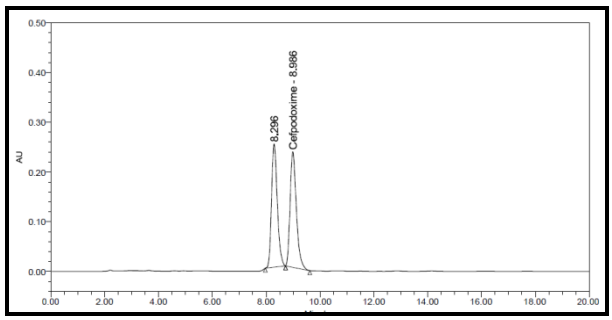

Figure 5: Chromatogram of Accuracy at 150%

Precision

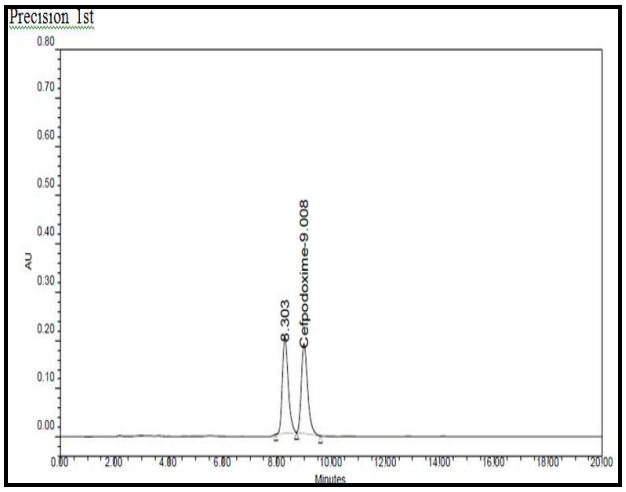

Figure 6: Chromatogram of Precision

Linearity

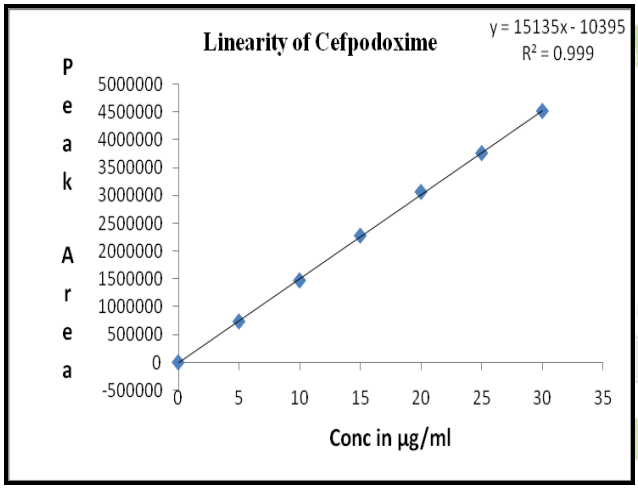

Figure 7: Linearity Graph of Cefpodoxime Proxetil

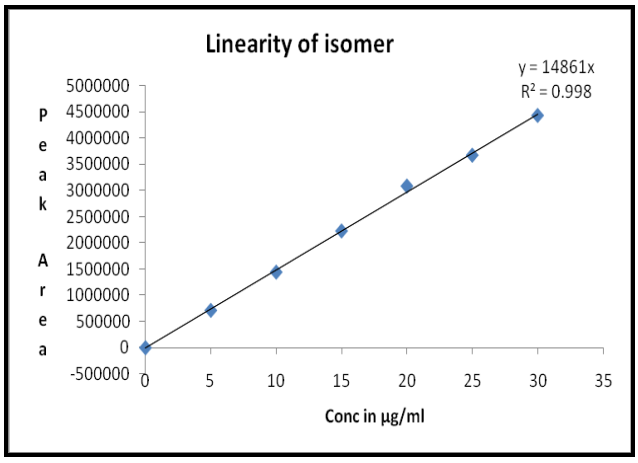

Figure.8: Linearity Graph of Isomer

Ruggedness

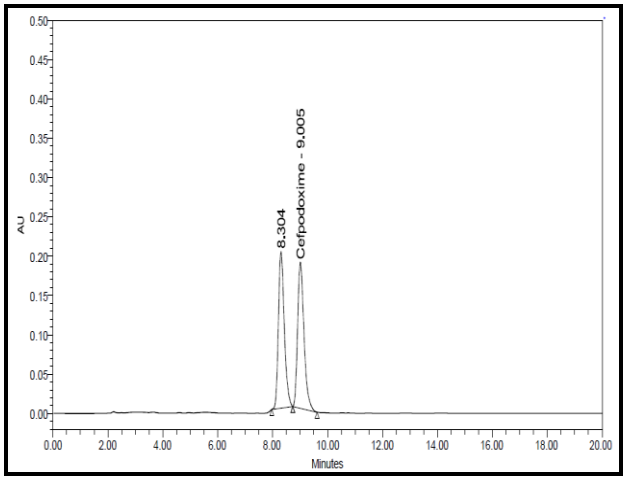

Figure 9: Chromatogram of Ruggedness

Robustness

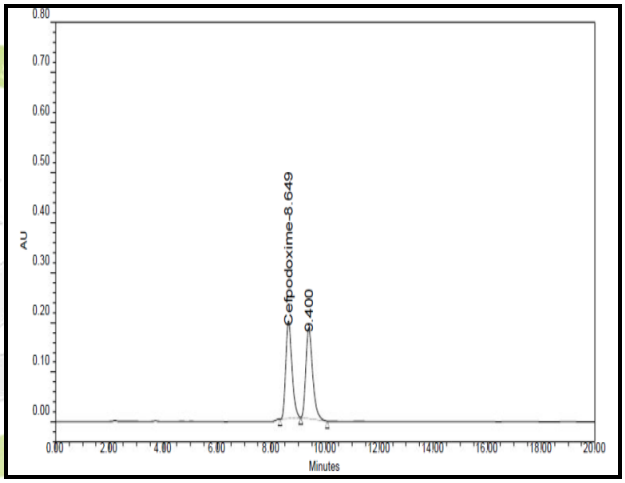

Figure 10: Chromatogram of Robustness

Degradation Methods

Acid

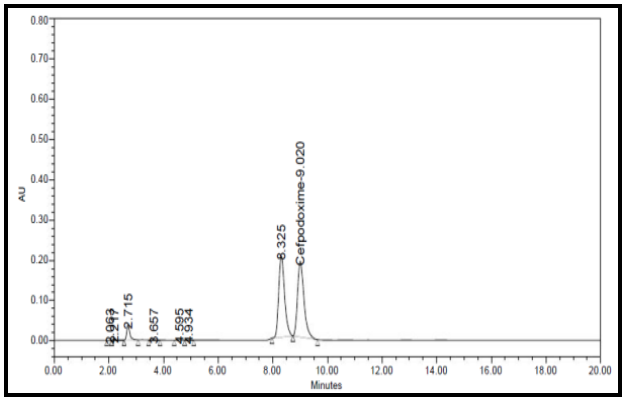

Figure 11: Chromatogram subjecting to Acid Condition

Base

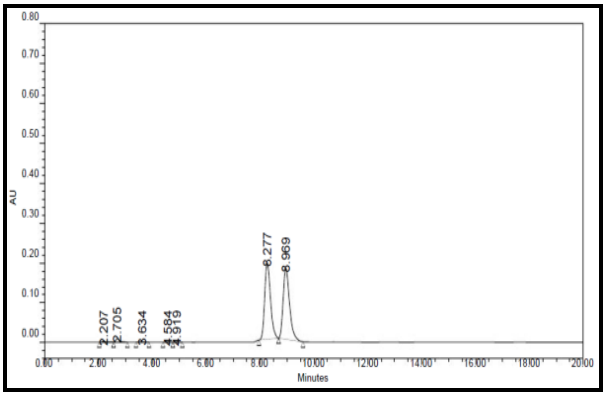

Figure 12: Chromatogram subjecting to Base Condition

Heating

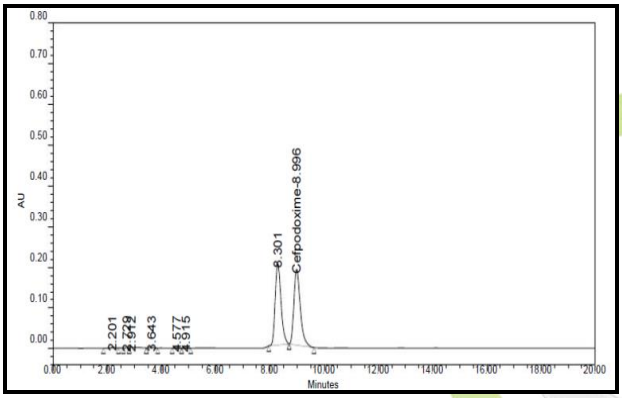

Figure 13: Chromatogram subjecting to Heating Condition

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